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Effect of acetonitrile on the hydration of human serum albumin films: a calorimetric and spectroscopic study

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Abstract

A new experimental approach based on the combination of calorimetric and FTIR spectroscopic measurements was proposed to study simultaneously the sorption of water and organic solvent, and corresponding changes in the structure of protein films in the water activity range from 0 to 1.0. Enthalpy changes (ΔH_{tot}) on the interaction of water with the dried human serum albumin (HSA) in the presence and absence of acetonitrile (AN) have been measured using a Setaram BT-2.15 calorimeter at 298 K. Spectroscopic data on water and organic solvent vapor sorption by the HSA films and the corresponding changes in the protein secondary structure were determined by means of a Bruker Vector-22 FTIR spectrometer. By using a water activity-based comparison we characterised the effect of acetonitrile on the hydration and structure of the HSA films. Acetonitrile (AN) sorption isotherm resembles a smooth curve. HSA film binds about 250 mol AN/mol protein at the lowest water activities. As the water activity increases from 0 to 0.8, the sorption of AN gradually decreases from 250 to 150 mol AN/mol HSA. At $a_w > 0.8$, the sorption of AN sharply decreases to zero. Acetonitrile decreases markedly the water content at a given a_w. This behavior suggests that the suppression in the uptake of water is due to a competition for water-binding sites on the HSA films by acetonitrile. Changes in the secondary structure of HSA were determined from infrared spectra by analyzing the structure of amide I band. Acetonitrile increases the intensity of the 1654 cm^{-1} band that was assigned to the α-helix structure. Changes in the intensity of the 1654 cm^{-1} band agree well with the decrease in water uptake in the presence of AN. An explanation of the acetonitrile effect on the hydration and structure of the HSA films was provided on the basis of hypothesis on water-assisted disruption of polar contacts in the initially dried protein.

Keywords: Protein films; Protein hydration; Protein structure; Human serum albumin; Organic solvent; Acetonitrile; Sorption; Isothermal calorimetry; Interaction enthalpies; Fourier transform infrared spectroscopy (FTIR spectroscopy)

1. Introduction

It is well known that the interaction of water with proteins plays a key role in determining the structure and functions of proteins [1–3]. Knowledge of processes occurring upon hydration or dehydration of proteins is very important in various practical applications of proteins such as their use as biocatalysts [4–6], biosensors [7,8] and selective adsorbents [9,10] in low water organic solvents or as thin films in bionanotechnology [11]. Hence, for a better understanding of the intermolecular interactions and conformational rearrangements that occur upon hydration of solid proteins in various environments there is a clear need of the experimental methods by which both the thermodynamic and structural properties of the hydration process in the presence of some additives, including organic solvents, may be obtained simultaneously.

The interaction enthalpies of proteins with the water–organic mixtures might be a very informative property of the intermolecular interactions in the above mentioned systems. Calorimetry is a reliable method to determine quantitatively this thermodynamic property. For example, based on the calorimetric measurements two different mechanisms of the interaction of the hydrated human serum albumin with organic solvents were proposed [12–14]. It was found that in low water pyridine, dioxane,

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